

Antioxidant and Total Phenolic Testing of Ethanolic Extract of Lime and Kaffir Lime Peel Waste and Peel Off Mask Formulation

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ABSTRACT

The use of synthetic ingredients in cosmetics causes many side effects such as premature aging, skin irritation, acne, blackheads and so on. So it is necessary to develop cosmetics from natural ingredients, such as using citrus waste to make peel-off masks. Development of peel-off mask preparations using active ingredients from kaffir lime peel and lime peel waste which have antioxidant activity and total phenolic content. The preparation of peel-off masks from citrus peel waste was carried out by extracting phenolic compounds from citrus peels using the maceration method with ethanol solvent and continued by formulating peel-off mask preparations with PVP and PVA bases. Furthermore, the peel off mask preparation was tested which included organoleptical, pH, adhesion, spreadability and viscosity tests. The method used to analyze the total phenolic content using Folin Ciocalteu method, while the antioxidant activity analysis was tested with DPPH method. The results of this study showed that the measurement of total phenolic content by Folin Ciocalteu method resulted in a value of 862.05 µg/ml per 1000µg of extract, while the measurement of antioxidant activity by DPPH method using a spectrophotometer at a wavelength of 515.5 nm amounted to 63.13 µg/ml with a positive control of vitamin C of 50.17 µg/ml. The peel off mask developed has good physical properties with the value of the preparation obtained pH 6, the value of spreadability 10.35 ± 0.41 grams.cm/s, the value of stickiness 8.00 ± 0.00 seconds, the value of viscosity 2,110.07 ± 83.97 cPa

INTRODUCTION

One of the body's protective tissues, namely the skin, will be damaged with age (Yusharyahya, 2021). This is caused by other things that come from outside the body (Russell-Goldman & Murphy, 2020). Genetic factors, gene mutations, lifestyle, environment, and the influence of free radicals are some of the factors that can accelerate skin aging (Yusharyahya, 2021). The appearance of fine lines, dark spots, chapping, scaling, and dryness are signs of skin aging that

can be seen visibly (Zubaydah et al., 2020). WHO states that aging is not only physical old age, but also mental and social health, including happiness and self-satisfaction, which can be achieved through the prevention and treatment of skin aging (Reza Fahlevi, Ni Deak Made Santi Diwyarthi, Dito Anurogo, Muhammad Anwari, 2023). Free radicals from harmful ultraviolet radiation are the main cause of premature aging in the form of photo oxidation, photo isomerase, and chemistry (Wahyono et al., 2011).

What can be done to prevent further oxidative stress reactions is the use of antioxidants (Muna, 2022). When the concentration of free radicals increases, the skin's natural system cannot protect skin cells from damage caused by free radicals (Euis, 2017). One way that can be done is to use topical skin care that contains antioxidants. The mechanism of antioxidant compounds by neutralizing free radicals, singlet oxygen molecules, topical antioxidant compounds protect cell membranes from oxidative stress (Muna, 2022). Many cosmetic preparations are developed with antioxidant content with topical use because of their broad biological activity mechanisms (Haerani et al., 2018). This is because in addition to having a mechanism in the protection of oxidation reactions but also functions as anti-inflammatory and anticarcinogenic (Haerani et al., 2018). This is because in addition to having a mechanism in the protection of oxidation reactions but also functions as anti-inflammatory and anticarcinogenic (Patricia, 2007). Antioxidants exist inside and outside the body naturally. One source of exogenous antioxidants is plants that have flavonoid secondary metabolites and vitamin C (Nurkhasanah et al., 2023). Flavonoids belong to the phenol class, which is the most common secondary metabolite in nature. Flavonoids also function as metal barriers and electron donors to keep reactive free radicals stable. In addition, flavonoid compounds have the ability to capture free radicals or stop oxidation reactions, which allows stopping or preventing free radical reactions (Sayuti, Kesuma, 2015).

Plants that contain flavonoids and vitamin C are *Citrus hystrix* Peel and *Citrus aurantifolia* Peel waste (Permadi et al., 2021). Many people have not utilized the skin waste from these fruits, even though the content of active substance compounds that act as antioxidants is very high in the fruit peel waste (Kurniawan & Deglas, 2019). *Citrus hystrix* contains various active compounds, including phenolic compounds and flavonoids. HPLC analysis of the aqueous extract of *C. hystrix* peel revealed the presence of gallic acid, catechins, caffeic acid, rutin, and quercetin (Ratanachamngong et al., 2023). Quercetin is a flavonoid class compound found to be the most active compound in aqueous extracts. This compound is known for its antioxidant properties and potential health benefits,

including anti-inflammatory, anti-infective, and neuroprotective effects (Piluzza G, 2011). Research conducted by (Ratanachamngong et al., 2023) showed that the water extract of *C. hystrix* skin showed antioxidant activity in capturing free radicals with the DPPH test method getting IC_{50} data of 14.91 mg/mL. In addition, antioxidant activity testing with the NO test obtained IC_{50} results of 4.46 mg/mL. The results of this study showed strong antioxidant activity of *C. hystrix* water extract. Meanwhile, *Citrus aurantifolia* contains compounds identified in lime peel extract, namely naringin, poncirin, and neoponcirin, which are flavonoid compounds that have a role as effective antioxidants. Research conducted by (Permadi et al., 2021) that the antioxidant activity of ethyl acetate extract peel with IC_{50} value of 457.6 ppm. This shows that lime peel extract shows strong antioxidant properties compared to other citrus fruits.

A type of preparation that can be developed to transport antioxidant compounds when used topically is the use of removable peel off gel mask preparations (Pratiwi & Wahdaningsih, 2018). This type of occlusive mask facilitates the penetration of active substances into the facial skin by maintaining moisture (Velasco MV, Vieira RP, Fernandes AR, Dario MF, Pinto CA, Pedriali CA, Kaneko TM, 2014). Peel-off gel masks are also beneficial because they are easy to remove like an elastic membrane, so they can be used easily and are painless when removed (Sulastri & Chaerunisaa, 2018). This mask can also help solve various skin problems, such as acne, large pores, wrinkles, and premature aging (Kartika, 2010). In addition, this mask can refresh, moisturize, soften, and cleanse facial skin (Vieira et al., 2009). Peel-off gel masks usually contain film formers, gelling agents, humectants, and other ingredients to improve the physical performance of the preparation and the aesthetic value of the preparation (Sulastri & Chaerunisaa, 2018). However, variations in the concentration and type of ingredients used can affect the performance and function of the preparation. Therefore, the best peel-off gel mask formulation process is essential to produce a peel-off gel mask preparation that is physically most effective for transporting antioxidant ingredients. The peel-off mask peels off as a thin plasticized film that leaves no residue behind. It is applied as a liquid film that is spread thinly across the face with fingers. It offers deep pore

cleansing, skin debris removal, and facial skin tightening, healing, and renewal. Additionally, peel-off can improve blood circulation by increasing the occlusive effect and offering a mild moisturizing benefit (Ajay B et al., 2023). Thus, the purpose of developing this peel off mask formula is to obtain a more stable mask preparation and increase user comfort because of the distinctive odor of the orange peel used (Linda et al., 2023). So in this study, the formulation process of peel-off gel mask preparations with active substances in the form of a combination of Citrus hystrix Peel and Citrus aurantifolia Peel waste waste will be carried out and analyzed for total phenolic content and antioxidant activity.

METHODS

Materials

The materials used in this study were Citrus hystrix Peel and Citrus aurantifolia Peel, 70% ethanol, hexane, ethyl acetate, distilled water, MgCl₂, FeCl₃, HCL, dragendorf reagent, benedict reagent, DPPH, Vitamin C, gallic acid, methanol, Na₂CO₃, folin ciocalteau reagent.

Tools

The tools used in this research are spectrophotometer, rotary evaporator, glassware, buchner funnel, vacuum pump, etc.

Preparation and ethanol extraction of Citrus hystrix Peel and Citrus aurantifolia Peel

The fruit peels were washed with running water, dried in an oven for 48 hours at 40 degrees Celsius, then pulverized with a blender into simplisia powder. Extraction was carried out using 70% ethanol with a ratio of (1:3) and tightly closed and left protected from light for three days times 24 hours. After three days, the filtrate was filtered and the remains were remacerated in the same way with 70% ethanol. After the macerate was collected, a rotary evaporator was used to separate the macerate until a concentrated extract was formed (Riski et al., 2021). How Qualitative Analysis Works (Dewi, 2020)

Flavonoid Test

10 ml of distilled water were added to each extract, and the mixture was then cooked on a

water bath. After filtering, it was dissolved in 1 ml of 96% ethanol containing magnesium powder or MgCl₂, and it was then dissolved in 10 ml of strong hydrochloric acid P. Flavones, chalcones, and aurones are indicated by a purple or yellow-red tint.

Alkaloid Test

After that, five milliliters of 2 N hydrochloric acid were added to each extract. After heating on a water bath for two minutes, three drops of Dragendorf LP reagent were added. If the results show a precipitate that is colored from orange yellow to brick red, then the sample contains alkaloids.

Saponin Test

For each extract, add 5 ml of distilled water. After that, it is heated for 5 minutes. After shaking for 5 minutes, the foam that forms approximately one centimeter high and remains stable after standing for ten minutes indicates the presence of saponins.

Tannin Test

Each extract was heated for 5 minutes after five ml of distilled water were added. 5 drops of 1% (b/v) FeCl₃ were then added to the filtrate after it had been filtered. Tannin compounds are indicated by the formation of a dark blue or greenish-black tint.

Vitamin C

Each extract was put into a test tube, and 15 drops of Benedict's reagent was added. The test tube was heated over low heat for two minutes. Check if a precipitate forms. A color ranging from yellowish green to brick red is a sign that vitamin C is elevated.

Antioxidant Testing of extract Citrus hystrix Peel and Citrus aurantifolia Peel

Preparation of extract stock solution

50 mg of sample extract was dissolved in a volumetric flask, stirred, and then added ethanol up to 50 mL to reach a concentration of 1 mg/ml.

Preparation of 50µM DPPH solution

50µM DPPH powder was weighed as much as 5 mg, dissolved to 250 mL with ethanol in a measuring flask.

Making vitamin C comparison solution

Weigh 5 mg of vitamin C and dissolve it with ethanol to make a 1mg/ml stock solution. Then mix the final volume to 5 ml of volumetric flask.

Calculating the absorption rate of DPPH blank solution

Pipette 3.8 mL of DPPH solution and 0.2 mL of ethanol into a volumetric flask until the volume reaches 5 mL. After homogenizing the solution, it was left for half an hour. Following that, a UV-Vis spectrophotometer set at 515 nm was used to detect the absorbance.

Measurement of extract activity

The concentration of fruit peel ethanol extract in the solution is 12.5, 25, 50, 100, and 150 µg/ml. After taking 0.2 ml of each, 3.8 ml of DPPH and ethanol were put to a 5 ml volumetric flask. The solution was then incubated at 37°C for 30 minutes in a dark environment. UV-Vis spectrophotometer set to 515 nm wavelength was then used to test the sample solution's absorbance.

Vitamin C activity measurement

Vitamin C solution was made in concentrations of 12.5; 25; 50; 100; 150 µg/ml. Each was taken 0.2 ml, then, into a 5 ml volumetric flask, 3.8 ml DPPH was added and added with ethanol up to 5 ml. Then, this mixture was incubated for 30 minutes at 37°C. The measurement was done by UV-Vis spectrophotometer with a wavelength of 515 nm (Muna, 2022).

Total Phenolic Test

Preparation of Gallic Acid Master

Solution Weighing as much as 10 mg of gallic acid, dissolve it in 10 ml of distilled water (1000 µg/mL).

Preparation of 10% Na₂CO₃ Solution

10 grams of Na₂CO₃ was weighed and then dissolved in 100 ml of distilled water.

Determination of Wavelength

For 1 ml of gallic acid solution with a concentration of 100µg/ml, 1 ml of Folin-Ciocalteu reagent was added, then mixed and allowed to stand for 3 minutes. Next, 3 ml of 10% Na₂CO₃ was added to the solution and allowed to stand at room temperature until the absorbance was measured.

Preparation of Gallic Acid Calibration Curve with Folin-Ciocalteu Reagent.

Test tubes were pipetted with 1 ml, 1.25 ml, 1.5 ml, and 1.75 ml of gallic acid mother liquor (1 mg/ml). Fill each test tube with 10 cc of distilled water. To 0.2 ml of sample per person, add 1 ml of Folin-Ciocalteu and 15.8 mL of distilled water. Then shake and wait for 8 minutes. 3 milliliters of 10% Na₂CO₃ were then added, carefully mixed, and allowed to stand for two hours. The absorbance was calculated using the maximum wavelength 765 nm.

Determination of Total Phenol content in Orange Peel Extract by Folin-Ciocalteu Method

50 milligrams of orange peel extract was weighed and then mixed in 50 ml of ethanol (1 mg/ml) to create orange peel extract at a concentration of 1 mg/ml. 0.2 mL of the sample extract solution (200 ppm) was pipetted, followed by 15.8 mL of distilled water and 1 ml of Folin-Ciocalteu. The mixture was then agitated until it was smooth. The solution was then left to remain at room temperature for two hours after adding 3 ml of 10% Na₂CO₃. The absorption was measured at 765 nm. The phenol content can be computed in milligrams of gallic acid equivalent per 100 grams of sample because the measurements were made three times (Suryanto & Wehantouw, 2009).

Preparation and evaluation of Peel Off Mask cosmetic preparation

Table 1. Formulation of Peel Off Mask 100 gram

No	Ingredient Name	Percentage (%)
1	Aquadest	62.98
2	PVA	11.30
3	PVP	0.87
4	Glycerine	3.43
5	Allantoin	0.87
6	Orange waste extract	1.74
7	Methyl paraben	0.87
8	Alcohol	17.39
Total		100.00

In the process of making peel off mask preparations, first dissolve PVA, PVP, Alantoin and glycerin which have functions as a base with a little water and heated until dissolved at a temperature below 90°C until it thickens (**Table 1**) (Sulastri & Chaerunisaa, 2018). Next, add niacinamide, 95% alcohol and orange peel extract and stir until homogeneous at 250 rpm for 30 minutes with a magnetic stirrer. Add

preservatives to maintain stability, namely methyl parabens and stir until homogeneous (Wulansari et al., 2024). Next, test the physical properties of the preparation in the form of pH, adhesion test, spreadability, and viscosity.

RESULT AND DISCUSSION

Citrus hystrix Peel and Citrus aurantifolia Peel waste can be utilized in making cosmetics because it contains various bioactive compounds such as phenolics and antioxidants that are beneficial to the skin (Indrastuti & Aminah, 2020). Citrus hystrix Peel and Citrus aurantifolia Peel is extracted using the maceration method. The maceration method allows the solvent to effectively extract active compounds from Citrus hystrix Peel and Citrus aurantifolia Peel (Kawiji et al., 2015). Maceration of citrus peel waste was carried out using 96% ethanol solvent. The use of this solvent is based on the fact that 96% ethanol can extract bioactive compounds from Citrus hystrix Peel and Citrus aurantifolia Peel effectively because it contains many flavonoid compounds, vitamin C, polyphenols that can act as antioxidants (Marfu'ah et al., 2020). The maceration process was carried out by remaceration to prevent the solvent from saturating so that it can effectively take active substances (Susanty & Bachmid, 2016). After the process of concentrating the extract using a rotary evaporator, the temperature is kept below 70 °C to prevent the destruction of active compounds (Haryanto, 2023). The extract that has been formed is tested for qualitative analysis to determine the content of secondary metabolites contained in orange peel extract.

Table 2. Qualitative testing results of citrus extract

No	Testing	Color formed	Result
1	Flavonoids	Yellow	+
2	Alkaloids	Orange Yellow	+
3	Saponins	Foam	+
4	Tannins	Deep blue black	+
5	Vitamin C	Brick red precipitate	+

Description: + results indicate the presence of secondary metabolite compounds

The test results show that orange peel extract contains compounds that act as antioxidants because it shows positive results (Table 2). Furthermore, antioxidant activity testing was carried out using the DPPH method which was read by a spectrophotometer at a wavelength of 515 nm. The principle of the DPPH method is a

method to measure the antioxidant capacity of a compound or extract through the inhibition of free radicals (Setiawan et al., 2018). This method is based on the nature of DPPH which is a stable free radical with a characteristic purple color, which can turn yellow when reduced by antioxidants (Wulan et al., 2019). The antioxidant activity measurement was compared with the positive control, vitamin C as positive control. The table of percent inhibition of DPPH by vitamin C is listed in Table 3.

Table 3. Inhibition of DPPH by Vitamin C

Concentration (µg/ml)	Absorbance	Percent inhibition (%)
12.5	0.657	1.203
25	0.494	25.714
50	0.232	65.113
100	0.037	94.436
150	0.033	95.038
Negative Control	0.665	0

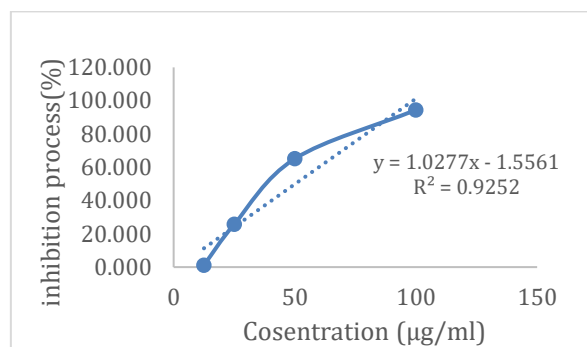


Figure 1. Linear regression curve of vitamin C positive control

The results showed that the positive control of vitamin C produced a linear regression curve equation $y = 1.0277x - 1.5561$ so that the IC_{50} of 50.17 µg/ml was obtained (Figure 1). The mechanism of vitamin C in acting as an antioxidant is that Vitamin C can reduce these free radicals into a less reactive or unreactive form, thus protecting cellular components from damage (Wibawa et al., 2020). This is because vitamin C can donate electrons to neutralize free radicals and other reactive molecules, thus preventing oxidative damage to cells and tissues (Fauzi, 2018). Furthermore, antioxidant testing of orange peel extract was carried out using the DPPH method. The test results are listed in Table 4.

Table 4. Inhibition of DPPH by orange peel waste extract

Concentration ($\mu\text{g/ml}$)	Absorbance	Percent inhibition (%)
12.5	0.472	21.070
25	0.395	33.946
50	0.218	63.545
100	0.137	77.090
150	0.094	84.281
Negative control	0.598	0

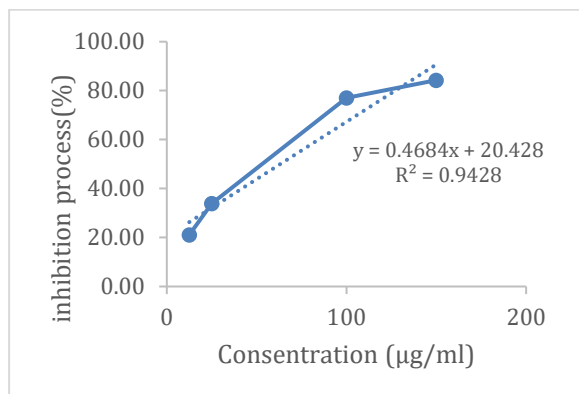


Figure 2. Citrus waste extract liner regression curve

The results showed that the Citrus hystrix Peel and Citrus aurantifolia Peel extract sample produced a linear regression curve equation $y=0.4684x+20.428$ so that the IC_{50} was obtained at $63.13 \mu\text{g/ml}$ (Figure 2). The content of active compounds contained in orange waste extract is polyphenolic compounds in the form of flavonoids, tannins, alkaloids (Ratanachamnung et al., 2023). Polyphenolic compounds of action as antioxidants is neutralizing free radicals by donating hydrogen atoms or electrons, converting free radicals into more stable and less reactive molecules (Dhianawaty & Ruslin, 2015). Polyphenols can play a role in the regeneration of other antioxidants such as vitamin C. After this vitamin neutralizes free radicals and becomes an oxidized form, polyphenols can reduce it back to its active form, thus prolonging antioxidant activity (Wibawa et al., 2020).

Furthermore, the total phenolic content was analyzed using the folin ciocalteau method. The principle of this method is the redox reaction between phenol in the sample with Folin-Ciocalteu reagent, which produces a color change that can be measured spectrophotometrically at a wavelength of 765 nm (Ryanata, 2015). The absorbance measurement results were compared with a standard curve made using pure phenolic compounds, such as gallic acid to determine the

total phenol content in the sample (Pramiastuti et al., 2018). The results of the gallic acid curve are listed in Table 5.

Table 5. Gallic acid standard curve

Concentration ($\mu\text{g/ml}$)	Absorbance
100	0.139
125	0.219
175	0.299
200	0.461

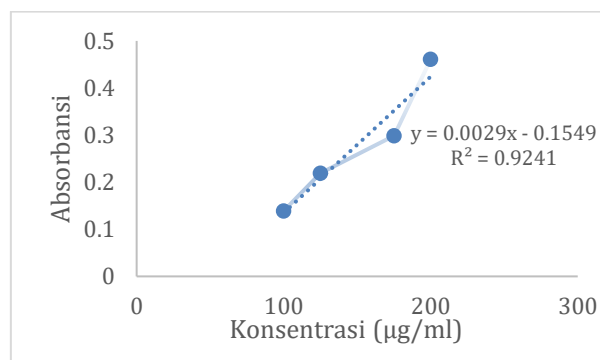


Figure 3. Gallic acid standard regression curve

The results showed that the standard sample of gallic acid produced a linear regression curve equation $y=0.0029x-0.1549$. Next, 0.2 ml of test sample ($200\mu\text{g/ml}$) was added to 15.8 ml of distilled water, 1 ml of folin digojok and allowed to stand for 8 minutes (Figure 3). Next, 3 ml of 10% Na_2CO_3 was added and the maximum wavelength was measured at 765 nm. The absorbance reading of the sample of Citrus hystrix Peel and Citrus aurantifolia Peel extract was done 3 times. The data of the sample Citrus hystrix Peel and Citrus aurantifolia Peel extract absorbance test results are 0.388; 0.332; 0.314 so that the average result is 0.345. Thus, the concentration of total phenolic content is $172.41 \mu\text{g/ml}$ per $200\mu\text{g}$ extract sample or $862.05 \mu\text{g/ml}$ per $1000\mu\text{g}$ extract. Testing of lime and kaffir lime peel waste extracts on antioxidant activity and total phenolic content produced good data. Furthermore, the preformulation of peel off mask preparations was carried out but no activity testing of the final product was carried out.

After testing the total phenolic content and antioxidant testing, then the orange peel extract is carried out in the process of making peel off mask preparations. The base used, PVA, has a function in forming a rigid and elastic film layer and provides strong adhesion so that the mask

will stick firmly (Amaliah et al., 2018). Meanwhile, PVP has the function of maintaining mask stability and retaining moisture so that the mask is easily removed (Sulastri & Chaerunisaa, 2018). PVP and PVA forms a flexible film, is easy to exfoliate, and provides a comfortable sensation compared to gelatin or carbomer (Ali & Kaid, 2024). Furthermore, glycerin and allantoin are added which have functions in maintaining skin moisture (Sukmawati et al., 2019). Glycerin has the advantage of being a natural moisturizer that is gentler and safer for sensitive skin than propylene glycol. In addition, glycerin is able to provide longer-lasting hydration due to its highly hygroscopic nature (Jang et al., 2015)

The preservative used in this study is methyl paraben which has the benefit of preventing microbial development in the preparation so that product stability also increases. The 96% alcohol solvent used has a role in helping the drying process of the film layer on the mask. If using ethanol below 96%, it affects the drying process of the preparation when applied to the skin to be a longer process (Zaujah et al., 2020)



Figure 4. Citrus Peel Off Mask Preparation

The results of this study indicate that the peel off mask preparation developed with active ingredients from kaffir lime and lime peel waste has good physical characteristics, such as an attractive greenish-yellow color, and has a smooth texture so that it can be easily applied to the skin (Figure 4). The main characteristics of the resulting peel-off mask product are the ability to effectively remove dead skin cells, moisturize, and reduce the appearance of pores (Widia et al., 2018). In this study, the peel-off mask produced is easy to apply to the skin and requires a short drying time of about 10 minutes and does not cause irritation to the skin (Figure 5).



Figure 5. Peel off mask when being applied to the skin

Based on quality-based design (QbD), the peel off mask developed from kaffir lime and lime peel waste proves that the preparation has good final quality based on ease of use application, drying time, ease of peeling, and stable preparation. This is because the peel off mask preparation developed has a suitable pH of 6.5, which shows that this preparation is suitable for application to the pH of the facial skin so that it does not cause irritation because the pH of cosmetic preparations for the face is in the range between 4.5-7.5 (Yumas, 2016). In addition, this peel off mask was tested for spreadability to help determine how easily the product can be applied and leveled on the skin (Lumentut et al., 2020). In this study, the spreadability value was obtained at 10.35 ± 0.41 grams.cm/s, then the adhesion test was carried out to determine whether a preparation product could stick to the skin (Lumentut et al., 2020). In this study, the adhesion value was obtained at 8.00 ± 0.00 seconds. As well as viscosity testing which has a function to measure the viscosity or flow resistance of a liquid or semi-fluid. In this study, the viscosity value was $2,110.07 \pm 83.97$ centipoise, which allows the peel off mask to produce a stable product.

CONCLUSIONS

Based on the research that has been done, it can be concluded that kaffir lime and lime peel waste can be utilized as the main active Ingredient in the manufacture of cosmetic peel off masks. Citrus peel waste has high phenolic content and strong antioxidant activity with an IC50 value of 63.13 $\mu\text{g/ml}$ with an IC50 value of vitamin C positive control of 50.17 $\mu\text{g/ml}$. In addition, the results of Citrus hystrix Peel and Citrus aurantifolia Peel extract the total phenolic content test were obtained at 862.05 $\mu\text{g/ml}$ per 1000 μg of extract. The bioactive

content in orange peel waste can support peel off mask preparations in treating the skin from free radical damage. The results of the physical evaluation of the preparation obtained pH 6, a spreadability value of 10.35 ± 0.41 grams.cm/s, an adhesive value of 8.00 ± 0.00 seconds, a viscosity value of $2,110.07 \pm 83.97$ centipoise. The use of orange peel waste also provides added value by utilizing previously unused

materials, thus supporting the concept of sustainability and environmental friendliness in the cosmetics industry.

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